PANETH CELL GRANULE OF MOUSE INTESTINE

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The Paneth cell secretion of the mouse intestine has been reported to be an acid mucopolysaccharide-protein complex; tryptophan as well as alpha acylamido carboxyl, amino, and phenolic groups of protein have been localized to the characteristic granules by means of histochemical staining procedures (13). Sulfhydryl and disulfide groups of protein have also been localized to the granule (14). The protein nature of the granule was suggested earlier by Hintzche and Anderegg (5).

Hally (3) concluded that the ultrastructure of the Paneth cell was typical of an exocrine, serozymogenic cell, and suggested that the secretory granule arose within the Golgi complex. It was noted that as the granule increased in size it became contained within a vacuole which appeared as a "halo" about the granule. The vacuole was not a constant feature, however, as there were occasional cells in which the space surrounding the granule was filled with a moderately osmiophilic material. The size of the vacuole was unaltered by changes in the tonicity of the fixative or washing fluid. Hampton and Quastler (4) also observed the granules of the mouse Paneth cell to be contained within vacuoles. Leblond (8) described an achromatic rim about the Paneth cell granule of the rat intestine following the PAS-staining procedure. Kurosumi (7) has suggested that the "halo" is produced by an extraction of a peripheral substance about the granule during preparation of the specimen.

The purpose of this investigation was an attempt to determine the chemical nature of the characteristic "halo" about the secretory granules of the

This investigation was supported by research grant A-1230 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.
Dr. Selzman was a trainee, United States Public Health Service Training Grant CRTY-5021.

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mouse Paneth cell by utilizing thin paraffin sections and various cytochemical staining techniques.

METHODS AND MATERIALS

Adult male mice with free access to food and water were killed by cervical dislocation. Pieces of small intestine 17 cm from the pylorus were removed and fixed in 10 per cent neutral buffered formalin for 24 hours. Tissues were washed for 1 hour in distilled water, dehydrated in graded alcohols for 6 hours, cleared for 3 hours in benzene, and embedded in filtered Tissue Mat (56°C M.P.). Sections were cut at 2 μ. The histochemical methods used to identify specific end-groups of proteins, including amino, carboxyl, phenolic, and sulfhydryl/disulfide groups, as well as procedures for the localization of polysaccharides, particularly acid mucopolysaccharides, were those previously employed (13, 14). A combination Alcian Blue-Periodic Acid-Schiff technique (10) was also carried out as an additional procedure for the identification of acid mucopolysaccharides.

RESULTS

The granules, as demonstrated by the presence of the various end-groups of protein, characteristically were surrounded by a conspicuous clear "halo" (Fig. 1). Alcian Blue alone did not stain the protein-rich granules, but specifically stained an outer rim, around each granule, comparable in size to the "halo" observed in the protein-staining procedures (Fig. 2). With the combination Alcian Blue-PAS technique, the protein component of the granule stained a characteristic magenta color (PAS-positive) and was surrounded by a rim of bluish-purple–stained material (Fig. 3). The latter color is considered to be specific for acid mucopolysaccharide (10). The granules were stained following the PAS technique (Fig. 4). Some granules revealed the characteristic "halo" with this procedure; others showed a more intense staining reaction about the periphery of the granule.

The largest granules, as demonstrated by the protein-staining procedures, were 2 μ in diameter; the vacuoles containing these granules were 3 μ in diameter. Specimens studied with the electron microscope gave values of 1.5 and 2 μ, respectively (3). Measurements of the granules and vacuoles permitted the deduction that the rim of acid mucopolysaccharide material could be superimposed upon the "halo" surrounding the protein-rich granules.

DISCUSSION AND CONCLUSIONS

It was concluded from our earlier study that the Paneth cell secretory granule in the mouse intestine was an acid mucopolysaccharide-protein complex (13). This concept must be slightly modified in view of the present findings. First, the acid mucopolysaccharide component can be morphologically separated from the protein-rich granule; that is, the clear "halo" demonstrated about the granules following procedures specific for protein end-groups is found to stain with methods considered specific for acid mucopolysaccharides. Second, the protein-rich granules are also stained with the PAS technique. Relative to the second point, Leblond *et al.* (9) concluded that only carbohydrate-protein complexes will give a positive reaction with the PAS technique if glycogen has been removed, and it has been shown that the PAS-positive component of the granule is resistant to diastase digestion (13). Therefore, it would seem reasonable to conclude that the Paneth cell secretory granule is a polysaccharide-protein complex surrounded by an acid mucopolysaccharide capsule.

The function of the Paneth cell secretion is unknown. It has been suggested that digestive enzymes, namely peptidases, are secreted by the Paneth cells (2); and DeCastro *et al.* (1) concluded that the Paneth cell secretion of the ant bear was an enzyme-polysaccharide complex. Thus, an appealing hypothesis would be one that postulates that the acid mucopolysaccharide capsule about the protein-rich granule protects the cell from a self-digesting process. Evidence has been reported for the presence of an intracytoplasmic inhibitor of trypsin activity in the exocrine cells of the pancreas (15). An earlier report by Kunitz and Northrop (6) also described an inhibitor of trypsin activity in pancreas homogenates that was characterized as a polypeptide.

The secretory granules of the mouse pancreas do not have a "halo" comparable to that seen about the Paneth cell granules (3). Palade (11) has described small granules within sacs (cisternae) in the endoplasmic reticulum of the guinea pig pancreas, but the profile of the large, typical zymogen granule in this gland revealed only a thin, dense membrane surrounding the granule. Recently, a study of the ultrastructure of the mouse parotid gland demonstrated certain types of secretory granules which had a homogeneous central core surrounded by an electron-opaque

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rim (12). Perhaps efforts directed toward determining whether the presence of a "halo" about the Paneth cell granule indicates a dissolution process of the mature granule or a step in maturation, as suggested by Kurosumi (7), will reveal the basis for the apparent morphological uniqueness of the Paneth cell secretory process.

The excellent technical assistance of Mrs. Elizabeth Hopkins is greatly appreciated.

Received for publication, May 7, 1962.

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Figure 1

Section of mouse intestinal gland stained for phenolic groups of protein. Note presence of clear "halo" about protein-rich granules. × 900.

Figure 2

Section of mouse intestinal gland stained with Alcian Blue. Note presence of stained rim about unstained granule. × 900.

Figure 3

Section of mouse intestinal gland stained with a combination Alcian Blue-periodic acid-Schiff technique. Note darker staining rim about PAS-stained granules. × 900.

Figure 4

Section of mouse intestinal gland stained with periodic acid–Schiff technique. Note clear "halo" about some granules and darker-stained periphery about others. × 900.

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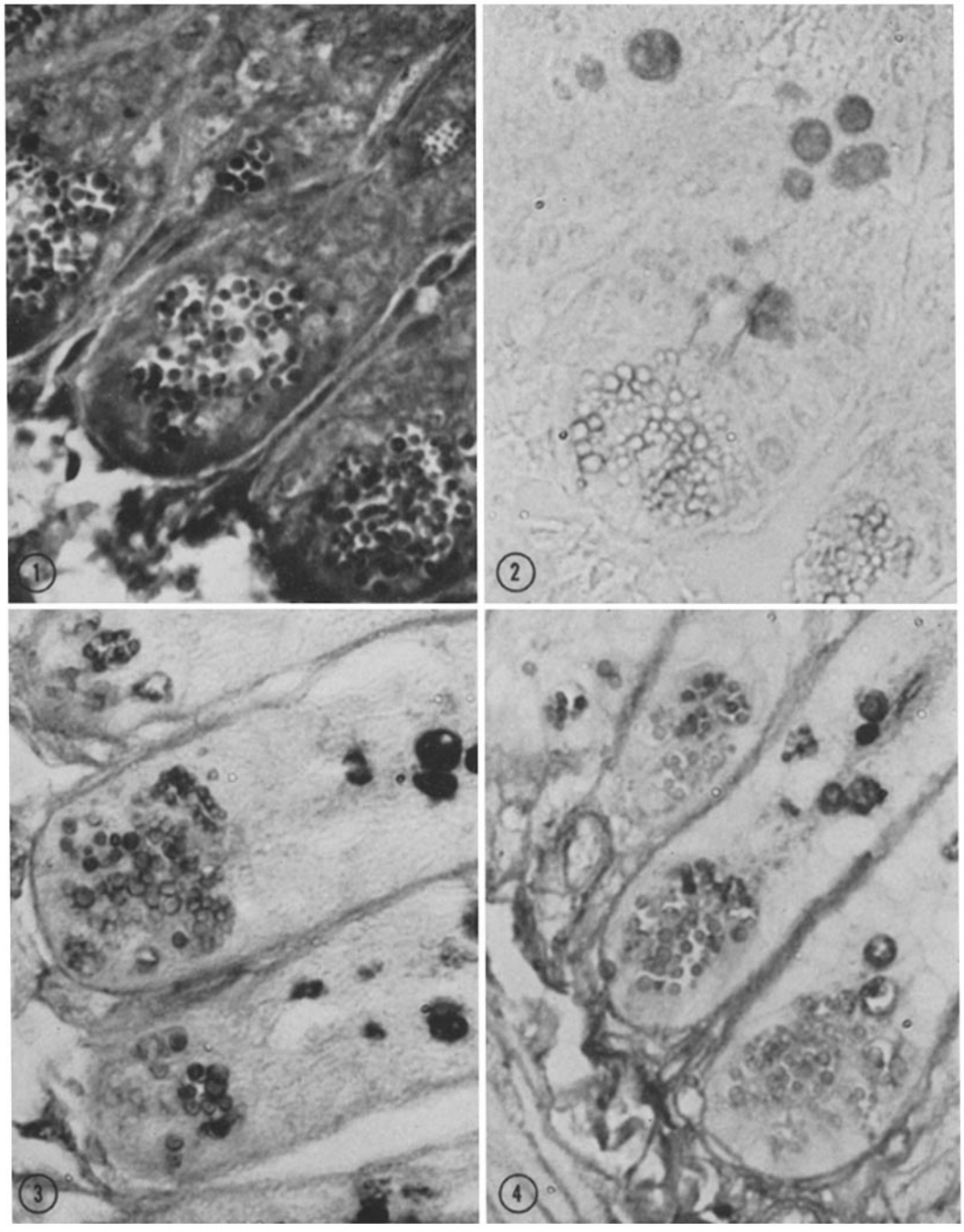

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